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STUDIES ON THE TOTAL INTAKE SIMULANT DMMPA,(U)
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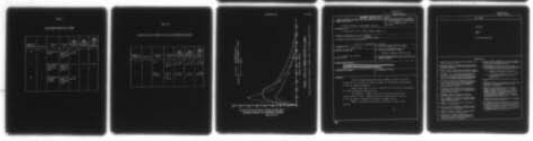

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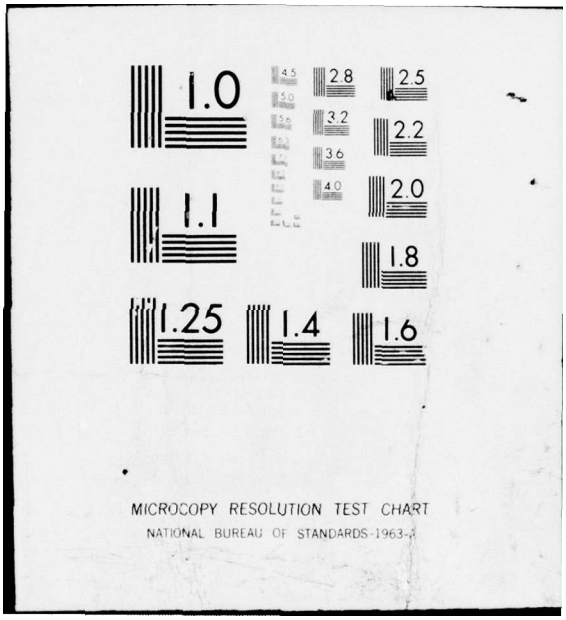
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SUFFIELD TECHNICAL PAPER

NO. 469

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(Unclassified version of STP 410 originally issued August 1973)

by

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ABSTRACT

Dimethyl morpholinophosphoramidate (DMMPA) has been tested for suitability as a simulant for chemical agents in studying the effectiveness of chemical defence procedures.

Results show that it is easily detected in urine after percutaneous, oral and intramuscular administration.

The compound is shown to have very low toxicity when administered acutely in mice. Twenty-eight days of application to the skin of rats and rabbits produced no evidence of damage to skin.

DMMPA is concluded to be useful and safe as a total intake simulant.

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INTRODUCTION

The search for a simulant which can be used to study the effectiveness of chemical defence procedures has been going on for several years. The concept of a total intake simulant for chemical agents has been developed at DRES. This is a compound that can be measured quantitatively in the urine after systemic absorption. Such compounds should be capable of being detected at the nanogram/ml level in urine.

The ratio of total intake of the simulant to the amount excreted in urine in 24 hours is first established by assaying for unchanged simulant in urine collected over a 24 hour period following an intramuscular injection. The amount recovered can be then expressed as a percentage of the amount injected. This figure can be used to determine the total intake by any other route simply by determining the amount of simulant in urine collected for 24 hours following exposure. One can then estimate the effect of such an intake had it been a toxic agent.

Benzyl salicylate was used in early experiments. The assay used was relatively insensitive. Consequently, a search was made for a compound which could be detected in extracts of urine in minute amount using gas chromatography and especially the Melpar Flame Photometric Detector (1), sensitive to molecules containing sulphur or phosphorus. Sulphur-containing cyclamate analogs were investigated but this approach was abandoned when human consumption of cyclamates was banned by the Food and Drug Directorate of the Department of National Health and Welfare.

The Chemistry Section at DRES prepared several compounds containing phosphorus and morpholine. It was first decided to use diethyl morpholinophosphoramidate (DEMPA) because the starting compounds required for its synthesis were readily available. Extensive testing was done on its use as a total intake simulant. The results obtained were encouraging and the preparation of several kilograms required for field trials was carried out (2). During the course of this preparation it was found that if any water was present in the reaction flask a toxic contaminant, triethyl pyro phosphate (TEPP), was formed. Because of this difficulty other esters of phosphoramidic acid were investigated.

One of these, dimethyl morpholinophosphoramidate (DMMPA), appeared to be suitable. Even though the starting compounds were more difficult to procure, the danger of producing a toxic preparation due to the formation of TEPP was abolished. This paper is a record of the testing of DMMPA as a simulant for a toxic compound which would have a vapour pressure between GF and VX.

MATERIAL

DMMPA was prepared by the method of Jardine and Hansen (3). The boiling point at 0.3 mm/Hg was 82^{o}C and the density at 25^{o}C was 1.216. The absolute viscosity at 25^{o}C was 0.1580 Stoke (4). The vapour pressure was 1×10^{-2} at 30^{o}C (5).

The stability of dilute solutions of DMMPA over a pH range was tested by keeping 10 samples of DMMPA in water in a water bath at 37^{o}C for one hour with frequent agitation. The pH of each sample was adjusted by the addition of 0.1N HCl to give a pH range from 6.63 to 2.00. The DMMPA content remained stable between pH 6.63 and pH 4.88. At pH 2.52 there was 33-1/3% loss in 1 hour and at pH 2.37 a 60% loss in 1 hour.

METHODS

Animal Experiments

The initial toxicity testing on a group of sixty female mice of the DRES strain showed the intraperitoneal LD_{50} of DMMPA to be 4.5 g/Kg

(SE ± 0.49). In comparison DEMPA has a toxicity of 1.75 g/Kg. Limited tests were carried out with 4 female New Zealand White rabbits and twelve 200 gm Wistar rats. Daily applications of neat DMMPA were applied to the skin of a shaved area just behind each animal's head. Two rabbits received 50 µl each, one 100 µl and the fourth 100 µl of propylene glycol as a control. Five rats received 20 µl of DMMPA, 5 received 50 µl propylene glycol as controls. Following a four week period of treatment the animals were sacrificed and pathological studies were made on the treated skin and essential organs. Within the limits of the tests used all the animals appeared to be completely unaffected by the dose applied. The skin under the site of application showed no evidence of histological change.

Assay Method

The method used was essentially the same as that used for DEMPA. However a new Microtek MT220 Gas Chromatograph* allowed an increase in sensitivity. The detector used was still the Melpar Flame Photometric Detector using the interference filter for isolation of the phosphorus emission at 526 mu. A Dual Pen Westronic MT22 Recorder** full scale 1 millivolt was used.

The chromatographic conditions were as follows:

Pyrex Column - 6 ft. in length and 1/4 inch O.D.
Packing - Chromasorb W, H.P., 80/100 mesh.
Liquid Phase - Silicone OV101, 10%.
Column Temperature - 225°C.
Flame Photometric Detector Temperature - 115°C.
Inlet Block - 190°C Outlet Block - 190°C.
Carrier Gas - Nitrogen Flow Rate - 90 ml/min.
Oxygen Flow Rate - 20 ml/min, 20 psi.
Air Flow Rate - 40 ml/min, 20 psi.

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* Microtek Division, Tracor Inc., 6500 Tracor Lane, Austin, Texas 78721, U.S.A.

** Westronics Inc., P.O. Box 11250, Fort Worth, Texas 76110, U.S.A.

Hydrogen Flow Rate - 200 ml/min, 30 psi.

Attenuation - 10^4 x 4.

The volumes of 24 hour urine samples were recorded and 1/2 ml toluene was added as a preservative to an aliquot of approximately 200 ml for refrigerated storage. The urine was assayed for DMMPA when convenient. It had been determined that there was no decrease in the DMMPA content under these conditions over a period of three months.

Chloroform was used to extract the urine as it could then be readily evaporated. The dried sample was taken up in n-hexane. Use of n-hexane reduced the number of interfering peaks in the GLC. The solubility of DMMPA in chloroform was a little more than 20% v/v. The solubility of DMMPA in n-hexane was about 3% v/v.

Twenty-five mls of each sample were pipetted into a 125 ml separatory funnel. To this was added 1 ml 5N NaOH and 35 mls of chloroform (certified ACS). The mixture was shaken vigorously for 1 minute and the phases were then allowed to separate for at least 20 minutes. The chloroform was drawn off into a 50 ml glass stoppered centrifuge tube and evaporated in a Buchler Rotary Evapo Mix* at approximately 25 mm of Hg and 35°C. To the residue was added 0.5 ml of n-hexane (certified ACS). The tubes were stoppered and agitated 15 seconds on a Vortex Jr. Mixer**. The n-hexane was ready for immediate injection. One microliter was injected into the GLC using a 1 µl Pressure-Lok syringe***. A four way valve was used to prevent the detector flame from blowing out. One minute was allowed for the solvent to vent before the effluent of the column was directed to the detector. The peak heights were measured in millimeters and the average height of 4 injections used to calculate the amount of DMMPA in the sample.

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* Buchler Instruments, New York 31, N.Y., U.S.A.

** Scientific Industries Inc., Queens Village, N.Y., U.S.A.

*** Precision Sampling Corporation, P.O. Box 15119, Baton Rouge, La. 70815, U.S.A.

An emulsion layer formed during the extraction process. This was different for each urine sample and a varying amount of DMMPA was thrown away. Therefore it was necessary to use internal standards: 0.5 nl (0.608 μg) of DMMPA in 1 ml of water was added to one of two aliquots of the same urine. Both samples were extracted in the same manner. Calculation for DMMPA content was as follows:

$$x = \frac{V_{TU}}{V_{AU}} \times W_D \times \frac{V_{TH}}{V_{IH}} \times \frac{H_S}{H_T - H_S}$$

x - quantity DMMPA in 24 hr. sample
V_{TU} - total volume of urine in 24 hr.
V_{AU} - volume of urine aliquot
W_D - quantity of added DMMPA in injected sample
V_{TH} - total volume of n-hexane
V_{IH} - volume of n-hexane injected
H_S - peak height for sample
H_T - peak height of sample plus added DMMPA

The reproducibility of the method was checked two ways:

(1) Ten analyses of one urine sample were made. This sample was of unknown content but the amount applied to the skin was known. The results showed a recovery of 17.3% (SD 1.19, SE 0.38) of the amount applied.

(2) Assays were performed on urine samples from each of five different individuals. Each sample was divided into three parts. To each part was added a known quantity of DMMPA (1, 2 or 3 μg). All samples were assayed for DMMPA content and the recovery was expressed as a percent of the amount added. The results are shown in Table I.

Human Studies

Skin absorption studies were done on 10 human volunteers from our laboratory. Each experiment was done in triplicate on the individuals

participating. The procedures were standardized to give accurate results and to cause minimal inconvenience to the volunteers.

(1) The volume applied was expressed from a microliter syringe, touched to the skin, and smeared over surface with the needle edge.

(2) The treated skin was left uncovered, but protected from exposure to weather or friction for the hour following the application.

(3) The skin area was washed one hour after treatment with a pad of kleenex soaked in water in such a way as not to wash any remnant of DMMPA into mouth or eyes.

(4) Urine was collected for one 24 or 48 hour period.

The figures in Table II are the unchanged DMMPA in the urine reported as a percentage of DMMPA applied to the skin.

In order to relate excretion to known VX toxicity data, intramuscular injections of 0.2 µl DMMPA in 0.2 ml saline were made in four human volunteers. These results are shown in Table III along with the results of the oral ingestion of 0.1 µl DMMPA in 1 ml water by four volunteers. The urine from these trials and a few additional percutaneous applications was collected in 12 hour periods of time for up to 96 hours so that rates of excretion were determined (Figure 1).

DISCUSSION

The same arguments for acceptability as an intake simulant for the chemical agent VX can be made for DMMPA as were made for DEMPA. The vapor pressure is within acceptable limits as it falls between those of GF and VX. The toxicity checks of the pure chemical as performed on mice and rats have shown the material to have a low acute systemic toxicity (5 g/Kg (i.p.) for mice and 4.2 g/Kg (oral) for rats) and innocuous by repeated percutaneous application over four weeks and, based on data available at this time, it is considered safe for specific experimental use on humans with certain restrictions as to routes and dosages. These restrictions which will be lifted as and when additional data becomes available are as follows:

Parenteral 1 mgm acute. (No more than four doses with at least three months between doses.)

Oral and percutaneous 1 mgm. (No more than 4 mgm per year.)

A rapid assay for minute quantities has been developed. Two nanograms of unchanged DMMPA per milliliter of urine were measured accurately. This was well within the limits of instrument sensitivity. DMMPA appears to be stable in urine. The DMMPA content remained constant in stored urine over a period of six months. Urine assayed for DMMPA after three, four and six month periods of refrigeration showed no decrease in DMMPA content.

Perhaps the most important characteristic required for DMMPA as a total intake simulant is its ability to be absorbed through skin in a similar manner to possible agents. This can be checked by examining the total intake of the simulant and the percutaneous LD_{50} of e.g. VX in man. There is a similarity between the skin penetration rates for VX and DMMPA.

Evidence from the studies of Sim (6) based on the 70% drop in human RBC cholinesterase value (CHE_{30}) and toxic symptomatology show the face-to-arm absorption ratio to be 8:1. If it is assumed that the amount of DMMPA in the urine reflects the amount absorbed by the skin, then the ratio of face-to-arm absorption of DMMPA is acceptable 4.6:1 (8.68:1.87) from Table II. Note here however that the reciprocal of the ratio is used because it is assumed that greater excretion means greater intake by absorption and therefore a lower LD_{50}.

Having all the excretion data at hand, Tables II and III, it is possible to estimate the hazard of exposure to an agent such as VX. If during exposure to this simulant the route of intake were known we would be able to estimate, from the urinary content of DMMPA, the lethality of a similar exposure to this agent. However, there may be several simultaneous routes of intake and an approximation of the total intake may be made by relating the 24 hour urinary content of simulant to the 24 hour excretion rate of DMMPA after an intramuscular injection. The calculated total intake can then be related to the intramuscular toxicity of the chemical agent.

It will be noted in Figure 1 that DMMPA is excreted in urine over a period of three days following its intake by any route; however, the 24 hour period was chosen as an arbitrary time for most experiments for convenience. In addition, the accuracy of an experiment using a large number of volunteers could diminish with time as the possibility of losing a urine sample increases with time. The results of the study indicate that DMMPA is a useful and safe compound for use as a total intake simulant.

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TABLE I

EFFICIENCY OF ASSAY

Individual	Volume Urine in ml	µg DMMPA Added	µg DMMPA Recovered	% Recovery
1	100	1	0.97	97
2	83	1	1.01	101
3	95	1	1.08	108
4	120	1	1.02	102
5	115	1	0.98	98
1	250	2	2.01	100.5
2	240	2	2.11	105.5
3	237	2	1.995	99.75
4	175	2	1.9	95
5	190	2	2.03	101.5
1	105	3	3.07	102
2	97	3	2.95	98
3	105	3	3.27	109
4	125	3	3.08	103
5	112	3	2.97	99

TABLE II

PERCUTANEOUS ABSORPTION OF DMMPA

Number of Volunteers	Exposures	Route of Entry	Solution Used	Mean % DMMPA Recovered in 24 hr	Mean % DMMPA Recovered in 48 hr	Mean % DMMPA Total 48 hr Recovery
10	3	Percutaneous Cheek	2 µl 10% DMMPA in propylene glycol	8.68 SE±0.74		
4	1	Percutaneous Cheek for excretion rate	5 µl 10% DMMPA in propylene glycol	9.37	5.55	14.92
10	3	Percutaneous Forearm (volar)	5 µl 10% DMMPA in propylene glycol	1.87 SE±0.47		

TABLE III

URINARY CONTENT OF DMMPA AFTER ORAL AND INTRAMUSCULAR INTAKE

Number of Volunteers	Exposures	Route of Entry	Solution Used	Mean % DMMPA Recovered in 24 hr	Mean % DMMPA Recovered in 48 hr	Mean % DMMPA Total 48 hr Recovery
4	2	Oral	0.1 µl in 1 ml water	17.58 SE±1.25	7.72 SE±1.23	25.30 SE±1.79
4	1	Intra-muscular	0.2 µl in 0.2 ml saline	16.58 SE±1.04	8.5 SE±0.74	25.08 SE±1.15

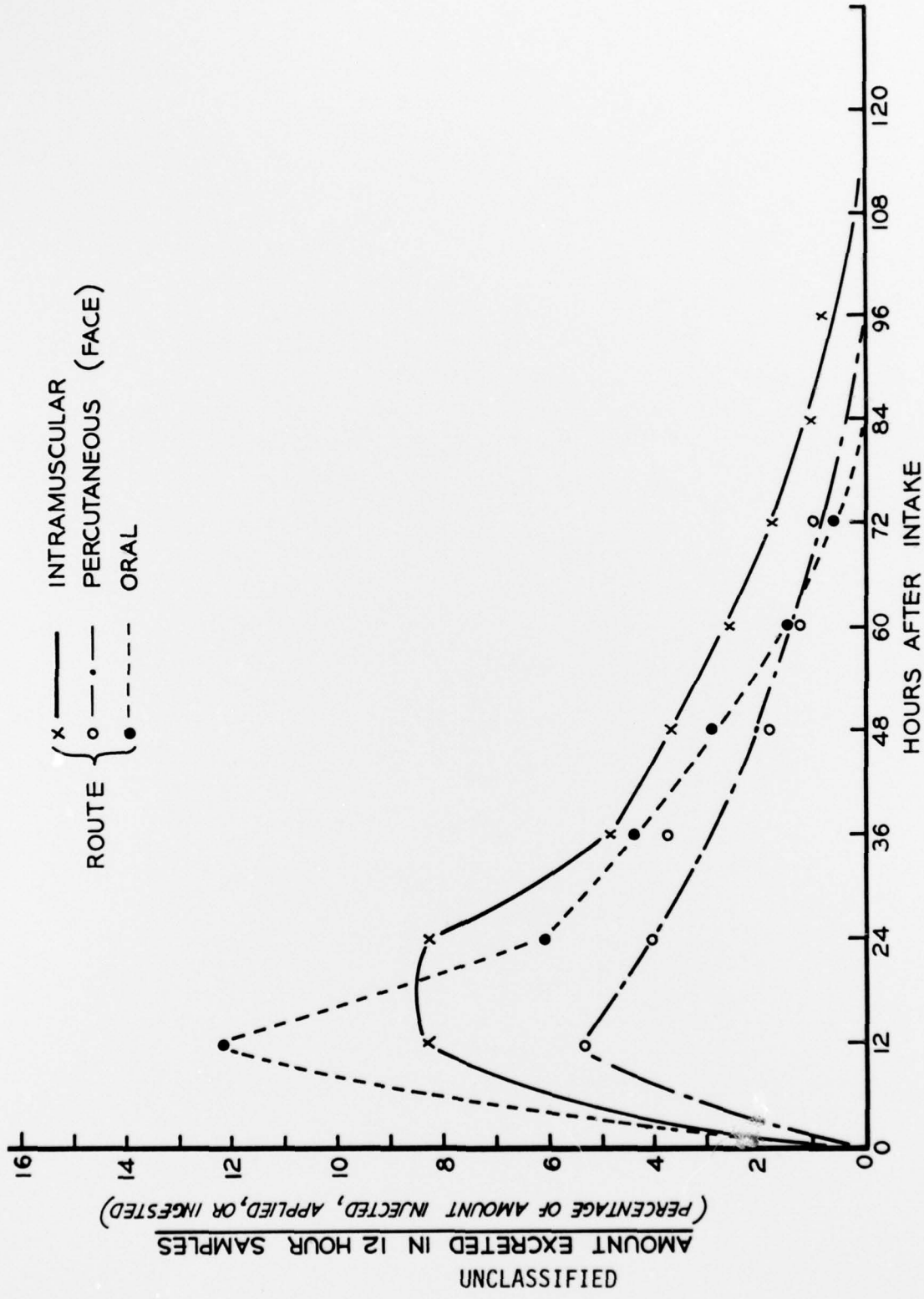

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FIGURE 1. RATES OF URINARY EXCRETION OF DMMPA

(EACH POINT INDICATES AMOUNT IN SAMPLE TAKEN FOR PREVIOUS 12 HOUR PERIOD.)

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